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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
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EXAMINER	
BOWMAN, AMY HUDSON	

ART UNIT	PAPER NUMBER
1635	

DATE MAILED: 09/01/2005

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/776,635	ROSSI ET AL.	
	Examiner	Art Unit	
	Amy H. Bowman	1635	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *21 July 2005*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-26* is/are pending in the application.

4a) Of the above claim(s) *3,5,9,10 and 26* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1,2,4,6-8 and 11-25* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☒ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *12 February 2004* is/are: a)☐ accepted or b)☒ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date *12/14/04*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

S.o.S

DETAILED ACTION

Applicant's election with traverse of group III, claims 1, 2, 4, 6-8, 11-22 and 23-25, in the reply filed on 7/21/2005 is acknowledged. Applicant asserts that all groups should be examined together because the claimed subject matter is directed to any gene of interest and not just to the arbitrary groups set forth by the examiner. Applicant further asserts that there is no undue search burden present. Applicant asserts that since the groups are classified in a single subclass, there is a lack of search burden. Applicant asserts that since the broad claim is drawn to any gene of interest, the claims should be examined together.

On the contrary, the broad claim will be examined and if found allowable, the genes will be rejoined. As explained in the office action mailed 6/28/05, there are claims that are drawn to separate and distinct types of genes, as well as claims drawn specifically to RASSF1. Each of the given target genes necessitate a separate and distinct search and examination. Additionally, although applicant has only relied upon the claims being drawn to any gene of interest as the basis for a lack of search burden, the claims are not only drawn to different types of target genes, but are also drawn to targeting different regions of the given gene, as well as to methods involving different outcomes (i.e. inactivating vs. activating a gene). Additionally, although each of the groups are classified in the same class and subclass, this class and subclass are large, encompassing a multitude of inventions that would each require a separate search and examination. Classification into the same class and subclass does not mean that any

invention within that class and subclass would necessarily return art against another invention.

The requirement for restriction is still deemed proper and is therefore made FINAL.

Claims 3, 5, 9, 10 and 26 are withdrawn from further consideration pursuant to 37 CFR 1.142(b), as being drawn to nonelected inventions, there being no allowable generic or linking claim. Applicant timely traversed the restriction (election) requirement in the reply filed on 7/21/2005.

Sequence Compliance

This application contains sequence disclosures that are encompassed by the definitions for nucleotide and/or amino acid sequences set forth in 37 CFR 1.821(a)(1) and (a)(2). However, this application fails to comply with the requirements of 37 CFR 1.821 through 1.825 because there are sequences on pages 14-16, 18 and 20 of the specification, as well as in figure 3 that do not contain a SEQ ID NO.

A complete response to this office action must correct the defects cited above regarding compliance with the sequence rules and a response to the action on the merits which follows.

The aforementioned instance of failure to comply is not intended as an exhaustive list of all such potential failures to comply in the instant application. Applicants are encouraged to thoroughly review the application to ensure that the entire

application is in full compliance with all sequence rules. This requirement will not be held in abeyance.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claim 22 is rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

The invention of the above claim is drawn the method for methylating a gene of interest of claim 1, wherein said gene is a RASSF1 gene.

At the outset, it is noted that the claim does not recite a specific target nucleotide sequence by SEQ ID NO, but rather refer to the broad genus of RASSF1 sequences.

The claims encompass a method of methylating any RASSF1 sequence, as well as encompass any RASSF1 homolog or allele from any species known or yet to be discovered of RASSF1, as well as DNA genomic fragments, spliced variants or fragment that retains RASSF1-like activity. Although the specification discloses siRNA sequences having complementarity to a RASSF1 sequence, the specification does not describe siRNA molecules directed to any other species of RASSF1 to describe the

instantly claimed genus of all RASSF1 genes. Each of the instantly disclosed siRNA molecules involved in the claimed method are targeted to a single sequence, although the claims are drawn to any RASSF1 sequence. One of ordinary skill in the art could not make such oligos to any RASSF1 without knowledge of the sequence. Given the breadth of sequences embraced in the instantly claimed genus, one could not envision the member oligonucleotides that target such a broad genus.

The scope of the claimed invention is broad and the skilled artisan would not be able to envisage the entire genus claimed of siRNA molecules that direct methylation of any RASSF1 RNA such that the skilled artisan would recognize that the applicant was in possession of the claimed genus at the time of filing.

Claims 1, 2, 4, 6, 7, 8, 11, 12, 13, 14, 15, 16, 17 and 19-25 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for exposing a cell to an siRNA molecule and screening for methylation *in vitro*, does not reasonably provide enablement for siRNA directed methylation *in vivo*. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention commensurate in scope with these claims.

The instant invention is drawn to a method for methylating a gene of interest in a mammalian cell comprising exposing said cell to an siRNA molecule which is specific for a target sequence in said gene of interest, wherein said siRNA directs methylation of said gene of interest, wherein said target sequence is located in a promoter region of

said gene of interest, wherein said mammalian cell is a human cell. The siRNA contains about 19-28 or about 21 base pairs. The invention is further drawn to transformation, transduction, transfection, or infection of said siRNA into the cell by introducing DNA sequences encoding a sense strand and an antisense strand of said siRNA, wherein said siRNA is expressed in the cell. The introduction is accomplished by using at least one vector, more specifically a plasmid vector, a viral vector, or an adeno-associated vector. The invention is further drawn to introduction via a liposome. The instant claims encompass *in vivo* effects.

The specification teaches introduction of siRNA via transfecting human cells with siRNA duplexes *in vitro*, followed by screening for DNA methylation.

The unpredictability of attenuating/inhibiting expression of a target gene in by RNA interference (RNAi) and the correlated outcome of DNA methylation is evident in post-filing art. For example, Mathieu et al. (Journal of Cell Science, 2004, 117(21), pp. 4884-4888) teach that the mechanism by which RNA signals are translated into DNA methylation imprints is currently unknown. Mathieu et al. teach that both RNAi and RNA-directed DNA methylation are both triggered by dsRNA, although the exact mechanism is not understood. Mathieu et al. teach that the simplest view is that siRNAs and/or their dsRNA precursors trigger methylation as well as RNAi. While it is recognized that introduction of dsRNA targeted to a specific gene may result in attenuation of expression of the targeted gene via RNAi, the degree of attenuation and the length of time that attenuation is achieved is not predictable. Since the effects of RNAi are unpredictable *in vivo*, the effects of the correlated DNA methylation are

considered unpredictable as well. Caplen et al. (Gene 2000, vol. 252, p.95-105) provides evidence of the unpredictability of dsRNA attenuation/inhibition of a targeted gene in vertebrate cells *in vitro*. Caplen et al. report that although dsRNA inhibits gene expression in cultured *Drosophila* cells, screening of three commonly used cell lines from three different species: human, hamster, and mouse, using cells expressing transgenes both transiently and permanently, produced mixed results. Transient transfection of dsRNA targeted to the βgal transgene into 293 and BHK31 cells resulted either in no effect (293 cells) or a non-specific decrease in gene expression (BHK21 cells). Transfection of dsRNA into mouse NIH/3T3 cells transduced with a retrovirus expressing βgal induced no detectable decrease in gene expression (see pages 102-103).

In order to practice the claimed invention *in vivo* a number of variables would have to be optimized, including 1). determining what sequences would constitute antisense sequences against the DNA sequences encoding a target gene and what antisense sequences would actually be effective at inhibiting expression of the target gene, 2). the form of the oligonucleotide, whether to use a modified oligonucleotide with one or more backbone, sugar or base modifications, 3). the mode of delivery of the oligonucleotide to an organism that would allow it to reach the targeted cell, 4). the amount of oligonucleotide that would need to be delivered in order to allow inhibition of the expression of a target gene once it reached the proper cell and 5). ensuring the oligonucleotide remains viable in a cell for a period of time that allows inhibition of the gene to an extent that there is a measurable and significant therapeutic effect. Each

one of these variables would have to be empirically determined for each oligonucleotide duplex.

Thus, while the specification is enabling for the *in vitro* examples set forth in the specification, the specification is not enabling for introducing any dsRNA for any target gene in any cell or animal as the art of attenuating gene expression by introducing dsRNA into a cell or organism is neither routine nor predictable. Thus, one of skill in the art could not practice the invention commensurate in scope with the claims without undue, trial and error experimentation and therefore, claims 1, 2, 4, 6, 7, 8, 11, 12, 13, 14, 15, 16, 17 and 19-25 are not enabled.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for a patent.
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.
>
> (e) the invention was described in (1) an application for patent, published under section 122(b), by another filed in the United States before the invention by the applicant for patent or (2) a patent granted on an application for patent by another filed in the United States before the invention by the applicant for patent, except that an international application filed under the treaty defined in section 351(a) shall have the effects for purposes of this subsection of an application filed in the United States only if the international application designated the United States and was published under Article 21(2) of such treaty in the English language.

Claims 1, 6-8 and 25 are rejected under 35 U.S.C. 102(b) as being anticipated by Elbashir et al. (Nature, 2001, Vol. 411, pp. 494-498).

The instant invention is drawn to a method for methylating a gene of interest in a mammalian cell comprising exposing said cell to an siRNA molecule which is specific for a target sequence in said gene of interest, wherein said siRNA is about 19-28 base pairs, more specifically about 21 base pairs, wherein said mammalian cell is a human cell and wherein said causes inactivation of said gene of interest.

Elbashir et al. teach a method of transfecting mammalian cells with siRNA duplexes (see page 495). Elbashir et al. teach that duplexes of 21 nucleotides RNAs mediate RNAi in cultured mammalian cells, including human embryonic kidney and HeLa cells. Elbashir et al. teaches inactivation of a target gene via RNA interference. Although the siRNA duplexes taught by Elbashir et al. are not disclosed as directing methylation of a target gene, the siRNA duplexes taught by Elbashir et al. meet the structural limitations of the instant claims and are therefore necessarily considered to function as instantly claimed. As stated in the MPEP (see MPEP 2112), something that is old does not become patentable upon the discovery of a new property. Elbashir et al. teaches siRNA duplexes meeting the structural limitations instantly claimed and teaches using such duplexes in the same method step as instantly claimed. Elbashir et al. teaches the only instantly recited active step being performed with the only instantly recited active compound. In order for the instant invention to be operative as claimed, Elbashir et al. would necessarily anticipate the instant invention.

Therefore, the instant invention is anticipated by Elbashir et al.

Claims 1, 6-8, 11-13, 18, 19 and 23-25 are rejected under 35 U.S.C. 102(a) as being anticipated by Miyagishi et al. (Nature Biotechnology, 2002, Vol. 19, pp. 497-500).

The instant invention is drawn to a method for methylating a gene of interest in a mammalian cell comprising exposing said cell to an siRNA molecule which is specific for a target sequence in said gene of interest, wherein said siRNA is about 19-28 base pairs, more specifically about 21 base pairs, wherein said mammalian cell is a human cell and wherein said causes inactivation of said gene of interest. The invention is further drawn to exposing the cell to the siRNA by introducing DNA sequences encoding a sense strand and an antisense strand of the siRNA, wherein the siRNA is expressed in the cell. The introduction is accomplished by transformation, transduction, transfection, or infection, and by using at least one vector, more specifically a plasmid vector, *in vitro*. The DNA sequences are in the same or are in separate vectors.

Miyagishi et al. teach U6 promoter-driven siRNAs that efficiently suppress targeted gene expression in mammalian cells. Miyagishi et al. teach that the U6 promoter directly transcribes small RNAs which can subsequently for siRNA duplexes in cells. Miyagishi et al. constructed a siRNA expression vector in which 19-nt sense and antisense sequences against a target gene were placed under the control of U6 promoters. Miyagishi et al. teach transfection of HeLa cells with the siRNA expression vector and further teach plasmid expression vectors. Although the siRNA duplexes taught by Miyagishi et al. are not disclosed as directing methylation of a target gene, the siRNA duplexes taught by Miyagishi et al. meet the structural limitations of the instant claims and are therefore necessarily considered to function as instantly claimed. As

stated in the MPEP (see MPEP 2112), something that is old does not become patentable upon the discovery of a new property. Miyagishi et al. teach siRNA duplexes meeting the structural limitations instantly claimed and teach a method comprising the same method step that is instantly claimed. Miyagishi et al. teaches the only instantly recited active step being performed with the only instantly recited active compound. In order for the instant invention to be operative as claimed, Miyagishi et al. would necessarily anticipate the instant invention.

Therefore, the instant invention is anticipated by Miyagishi et al.

Claims 1, 6-8, 11, 17-20 and 25 are rejected under 35 U.S.C. 102 (a) or (e) as being anticipated by Fire et al. (US 6,506,559 B1).

The instant invention is drawn to a method for methylating a gene of interest in a mammalian cell comprising exposing said cell to an siRNA molecule which is specific for a target sequence in said gene of interest, wherein said siRNA is about 19-28 base pairs, more specifically about 21 base pairs, wherein said mammalian cell is a human cell and wherein said causes inactivation of said gene of interest. The invention is further drawn to exposing the cell to the siRNA by introducing DNA sequences encoding a sense strand and an antisense strand of the siRNA, wherein the siRNA is expressed in the cell. The introduction is accomplished by transformation, transduction, transfection, or infection, *in vivo* or *in vitro*.

Fire et al. teach a method to inhibit expression of a target gene in an animal cell comprising introduction of RNA into the cell in an amount sufficient to inhibit expression

of the target gene, wherein the RNA is a double-stranded molecule with a first strand consisting essentially of a ribonucleotide sequence which corresponds to a nucleotide sequence of the target gene and a second strand consisting essentially of a ribonucleotide sequence which is complementary to the nucleotide sequence of the target gene, wherein the first and the second ribonucleotide strands are separate complementary strands that hybridize to each other to form said double-stranded molecule, and the double-stranded molecule inhibits expression of the target gene (see claims 1 and 6). Fire et al. teach that the cell with the target gene may be derived from a vertebrate such as a human (see column 8). Fire et al. teach that a regulatory region (promoter) may be used to transcribe the RNA strand or strands *in vivo* or *in vitro* (see columns 8 and 9). Inhibition may be targeted by specific transcription in an organ, tissue, or cell type. Fire et al. teach the use and production of expression constructs. Fire et al. teach that the duplex RNA is capable of hybridizing with a portion of the target gene transcript and that the length of the identical nucleotide sequences may be at least 25 bases (see column 8 and claim 10). Fire et al. teach that the organism can be transfected with the expression construct. Fire et al. teach compositions comprising the nucleic acid and a solution, lipid-mediated carriers, or chemical-mediated carriers (see column 9). Although the RNA duplexes taught by Fire et al. are not disclosed as directing methylation of a target gene, the RNA duplexes taught by Fire et al. meet the structural limitations of the instant claims and are therefore necessarily considered to function as instantly claimed. As stated in the MPEP (see MPEP 2112), something that is old does not become patentable upon the discovery of a new property. Fire et al.

teach RNA duplexes meeting the structural limitations instantly claimed and teach a method comprising the same method step that is instantly claimed. Fire et al. teaches the only instantly recited active step being performed with the only instantly recited active compound. In order for the instant invention to be operative as claimed, Fire et al. would necessarily anticipate the instant invention.

Therefore, the instant invention is anticipated by Fire et al.

Claims 1, 11-14, 18-21 and 23-25 are rejected under 35 U.S.C. 102(e) as being anticipated by Graham (US 6,573,099 B2).

The instant invention is drawn to a method for methylating a gene of interest in a mammalian cell comprising exposing said cell to an siRNA molecule which is specific for a target sequence in said gene of interest, wherein the method causes inactivation of said gene of interest. The invention is further drawn to exposing the cell to the siRNA by introducing DNA sequences encoding a sense strand and an antisense strand of the siRNA, wherein the siRNA is expressed in the cell. The introduction is accomplished by transformation, transduction, transfection, or infection, and by using at least one vector, more specifically a plasmid or viral vector, *in vitro*. The DNA sequences are generated by PCR and the introduction can be achieved via a liposome. The DNA sequences are in the same or are in separate vectors.

Graham teaches an isolated genetic construct and an animal cell comprising such a construct which is capable of delaying, repressing, or otherwise reducing the expression of a target gene in an animal cell which is tranfected with said genetic

construct, wherein said genetic construct comprises at least two copies of a structural gene sequence and each copy of said structural gene sequence is separately placed under the control of a promoter which is operable in said cell, and wherein said structural gene sequence comprises a nucleotide sequence which is substantially identical to at least a region of said target gene, wherein at least one copy of said structural gene sequence is placed operably in the sense orientation under the control of an individual promoter sequence, and wherein at least one other copy of said structural gene sequence is placed operably in the antisense orientation under the control of another individual promoter sequence (see claim 4). Graham teaches genetic constructs comprising a synthetic gene inserted into a suitable vector which is capable of being maintained and/or replicated and/or expressed in the host cell into which it is introduced (see column 13). Specifically, Graham teaches bacteriophage vectors, viral vectors and plasmids (see column 13), as well as PCR amplification of the DNA sequences. Graham teaches a composition comprising the genetic construct and a suitable carrier, such as a liposome (see column 13). Graham teaches transfection or transformation of the target cell. Although the genetic constructs taught by Graham are not disclosed as directing methylation of a target gene, the genetic constructs taught by Graham meet the structural limitations of the instant claims and are therefore necessarily considered to function as instantly claimed. As stated in the MPEP (see MPEP 2112), something that is old does not become patentable upon the discovery of a new property. Graham teaches genetic constructs meeting the structural limitations instantly claimed and teach a method comprising the same method step that is instantly

claimed. Graham teaches the only instantly recited active step being performed with the only instantly recited active compound. In order for the instant invention to be operative as claimed, Graham would necessarily anticipate the instant invention.

Therefore, the instant invention is anticipated by Graham.

Claims 1, 6-8, 11-15, 18, 19, 21, 23 and 25 are rejected under 35 U.S.C. 102(a) as being anticipated by Qin et al. (PNAS, 2003, Vol. 100, No. 1, pp. 183-188).

The instant invention is drawn to a method for methylating a gene of interest in a mammalian cell comprising exposing said cell to an siRNA molecule which is specific for a target sequence in said gene of interest, wherein said siRNA is about 19-28 base pairs, more specifically about 21 base pairs, wherein said mammalian cell is a human cell and wherein said causes inactivation of said gene of interest. The invention is further drawn to exposing the cell to the siRNA by introducing DNA sequences encoding a sense strand and an antisense strand of the siRNA, wherein the siRNA is expressed in the cell. The introduction is accomplished by transformation, transduction, transfection, or infection, and by using at least one vector, more specifically a plasmid or viral vector, *in vitro*. The vector is more specifically a lentiviral vector. The DNA sequences are generated by PCR and the introduction can be achieved via a liposome. The DNA sequences are in the same vector.

Qin et al. teach a method of inhibiting HIV-1 infection in human T cells by lentiviral-mediated delivery of siRNA against CCR5, wherein the siRNA duplex is approximately 21 nucleotides long (see abstract). Qin et al. teach amplification of the

DNA with primers. Qin et al. teach lentiviral vector transduction *in vitro*. Although the RNA duplexes taught by Qin et al. are not disclosed as directing methylation of a target gene, the RNA duplexes taught by Qin et al. meet the structural limitations of the instant claims and are therefore necessarily considered to function as instantly claimed. As stated in the MPEP (see MPEP 2112), something that is old does not become patentable upon the discovery of a new property. Qin et al. teach RNA duplexes meeting the structural limitations instantly claimed and teach a method comprising the same method step that is instantly claimed. Qin et al. teaches the only instantly recited active step being performed with the only instantly recited active compound. In order for the instant invention to be operative as claimed, Qin et al. would necessarily anticipate the instant invention.

Therefore, the instant invention is anticipated by Qin et al.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 1, 2, 4, 6-8, 22 and 25 are rejected under 35 U.S.C. 103(a) as being unpatentable over Elbashir et al. (Nature, 2001, Vol. 411, pp. 494-498), in view of Mette et al. (The EMBO Journal, 2000, Vol. 19, No. 19, pp. 5194-5201), further in view of Dammann et al. (Nature Genetics, 2000, Vol. 25, pp. 315-319).

The instant invention is drawn to a method for methylating a gene of interest in a mammalian cell comprising exposing said cell to an siRNA molecule which is specific for a target sequence in said gene of interest, wherein said siRNA is about 19-28 base pairs, more specifically about 21 base pairs, wherein said mammalian cell is a human cell and wherein said causes inactivation of said gene of interest. The target sequence is located in a promoter region. The invention is further drawn to exposing the cell to the siRNA by introducing DNA sequences encoding a sense strand and an antisense strand of the siRNA, wherein the siRNA is expressed in the cell. The introduction is accomplished by transformation, transduction, transfection, or infection, and by using at least one vector, more specifically a plasmid, viral, or adeno-associated vector, *in vitro*. The vector is more specifically a lentiviral vector, a retroviral vector or an adenoviral vector. The DNA sequences are generated by PCR and the introduction can be achieved via a liposome. The DNA sequences are in the same or separate vectors and the gene of interest is a RASSF1 gene.

Elbashir et al. teach a method of transfecting mammalian cells with siRNA duplexes (see page 495). Elbashir et al. teach that duplexes of 21 nucleotides RNAs mediate RNAi in cultured mammalian cells, including human embryonic kidney and HeLa cells. Elbashir et al. teach inactivation of a target gene via RNA interference. Although the siRNA duplexes taught by Elbashir et al. are not disclosed as directing methylation of a target gene, the siRNA duplexes taught by Elbashir et al. meet the structural limitations of the instant claims and are therefore necessarily considered to function as instantly claimed. As stated in the MPEP (see MPEP 2112), something

that is old does not become patentable upon the discovery of a new property. Elbashir et al. teaches siRNA duplexes meeting the structural limitations instantly claimed and teaches using such duplexes in the same method step as instantly claimed. Elbashir et al. specifically teach that DNA methylation has been linked to post transcriptional silencing in plants.

Elbashir et al. do not teach specifically targeting a promoter region or specifically targeting the RASSF1 gene.

Mette et al. teach that dsRNA induces a post-transcriptional gene silencing process, termed RNAi, in diverse organisms. Mette et al. teach that transcriptional gene silencing accompanied by de novo methylation of a target promoter in plants can be triggered by a dsRNA containing promoter sequences (see abstract). Mette et al. teach that RNAi is a post-transcriptional gene silencing mechanism in which dsRNA induces the degradation of homologous RNA sequences and that the relationship of various types of PTGS has been confirmed recently by the analysis of mutants which has revealed that these processes require some of the same gene products in different organisms (see Introduction, first paragraph).

Dammann et al. teach the cloning and characterization of a human RAS effector homologue (RASSF1). Dammann et al. identified three transcripts, A, B and C. Loss of expression was correlated with methylation of the CpG-island promoter sequence of RASSF1A. Dammann et al. teach that RASSF1A has a potential role as a lung tumour suppressor gene. Dammann et al. teach experiments to study the effect of RASSF1A expression on cell growth.

It would have been obvious to one of ordinary skill in the art to design a siRNA to inhibit the expression of a target gene as taught by Elbashir et al. Further, it would have been obvious to one of ordinary skill in the art to expose a cell to a siRNA which is specific for a target sequence in order to methylate a gene of interest in a mammalian cell since Mette et al. teach the process of introducing siRNA duplexes to induce RNAi, and the RNAi is accompanied by *de novo* methylation of a target promoter in plants. Mette et al. teach the correlation between RNAi and PTGS and that each of the processes require some of the same gene products in different organisms. It would have been obvious to introduce a siRNA to methylate a RASSF1 to study the genes function because Dammann et al. teach that methylation of RASSF1 is correlated to lung tumors and that RASSF1A may have a potential role as a lung tumor suppressor gene.

One would be motivated to design a siRNA, as taught by Elbashir et al., to methylate a gene of interest, as taught by Mette et al, and to specifically target and methylate a RASSF1 gene, as taught by Dammann et al., with the motivation of studying a RASSF1 genes role in lung tumors since RASSF1 methylation is associated with lung tumors.

Finally, one would have a reasonable expectation of success given that Elbashir et al. teach designing siRNA molecules to direct cleavage of known genes and Mette et al. teaches the correlation between PTGS and RNAi, which is linked to DNA methylation. One would reasonably expect for the method of Elbashir et al. to be applicable to a known gene, such as RASSF1.

Thus in the absence of evidence to the contrary, the invention as a whole would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Amy H. Bowman whose telephone number is 571-272-0755.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Andrew Wang can be reached on 571-272-0811. The fax phone number for the organization where this application or proceeding is assigned is 703-872-9306.

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